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6. AUTHORS
Caroline S. Harwood

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5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAMES AND ADDRESSES
University of Washington
Box 359472
4333 Brooklyn Ave NE
Seattle, WA 98195 -9472

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14. ABSTRACT
Many bacteria use homoserine lactone (HSL) quorum-sensing (QS) signals to communicate and to control gene expression in a cell-density dependent manner. Durinh this project we completed a first set of fundamental studies of a new p-coumaroyl-HSL (pC-HSL) microbial communication system. This system of communication was novel when it was discovered because it is not based on fatty acid metabolism. We were also interested in identifying other non-fatty acid acyl-HSL systems in other bacteria. pC-HSL is detected by a signal receptor and transcription factor named RpaR. During the project period we: 1) published a study characterizing RpaR, 2) discovered and

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Report Title

ABSTRACT

Many bacteria use homoserine lactone (HSL) quorum-sensing (QS) signals to communicate and to control gene expression in a cell-density dependent manner. Durinh this project we completed a first set of fundamental studies of a new p-coumaroyl-HSL (pC-HSL) microbial communication system. This system of communication was novel when it was discovered because it is not based on fatty acid metabolism. We were also interested in identifying other non-fatty acid acyl-HSL systems in other bacteria. pC-HSL is detected by a signal receptor and transcription factor named, RpaR. During the project period we: 1) published a study characterizing RpaR, 2) discovered and published a description of an RpaR anti-sense RNA that inhibits rpaR translation. The cis-RNA represents a new layer of regulation that can be brought to bear on the activity of a QS system and 3) published a study describing a second aryl-HSL signal, cinnamoyl-HSL, produced by a photosynthetic Bradyrhizobium species. The cinnamoyl-HSL QS system operates at 1000-fold lower concentrations than do other QS systems. Certain features of this system allow Bradyrhizobium sp. to eavesdrop on other bacteria and to also avoid detection by other bacteria. These novel signals appear to control biofilm formation by the bacteria that produce them.

Enter List of papers submitted or published that acknowledge ARO support from the start of the project to the date of this printing. List the papers, including journal references, in the following categories:

(a) Papers published in peer-reviewed journals (N/A for none)

Received		Paper
04/12/2012	5.00	H. Hirakawa, A. L. Schaefer, E. P. Greenberg, C. S. Harwood. Anaerobic p-Coumarate Degradation by Rhodopseudomonas palustris and Identification of CouR, a MarR Repressor Protein That Binds p-Coumaroyl Coenzyme A, Journal of Bacteriology, (02 2012): 0. doi: 10.1128/JB.06817-11
08/29/2011	1.00	A. L. Schaefer, E. Giraud, E. P. Greenberg, N. A. Ahlgren, C. S. Harwood. Aryl-homoserine lactone quorum sensing in stem-nodulating photosynthetic bradyrhizobia, Proceedings of the National Academy of Sciences, (04 2011): 7183. doi: 10.1073/pnas.1103821108
08/29/2011	2.00	H. Hirakawa, Y. Oda, S. Phattarasukol, C. D. Armour, J. C. Castle, C. K. Raymond, C. R. Lappala, A. L. Schaefer, C. S. Harwood, E. P. Greenberg. Activity of the Rhodopseudomonas palustris p-Coumaroyl-Homoserine Lactone-Responsive Transcription Factor RpaR, Journal of Bacteriology, (03 2011): 2598. doi: 10.1128/JB.01479-10
08/31/2012	3.00	Federico E. Rey, Caroline S. Harwood. FixK, a global regulator of microaerobic growth, controls photosynthesis in Rhodopseudomonas palustris , Molecular Microbiology, (02 2010): 0. doi: 10.1111/j.1365-2958.2009.07037.x
08/31/2012	6.00	H. Hirakawa, C. S. Harwood, K. B. Pechter, A. L. Schaefer, E. P. Greenberg. Antisense RNA that affects Rhodopseudomonas palustris quorum-sensing signal receptor expression, Proceedings of the National Academy of Sciences, (07 2012): 0. doi: 10.1073/pnas.1200243109
TOTAL:	**5**	

Number of Papers published in peer-reviewed journals:

(b) Papers published in non-peer-reviewed journals (N/A for none)

Received Paper

TOTAL:

Number of Papers published in non peer-reviewed journals:

(c) Presentations

Number of Presentations: 0.00

Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received Paper

TOTAL:

Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received Paper

TOTAL:

Number of Peer-Reviewed Conference Proceeding publications (other than abstracts):

(d) Manuscripts

Received	Paper

TOTAL:

Number of Manuscripts:

Books

Received	Paper

TOTAL:

Patents Submitted

Patents Awarded

US patent #7,745,023, awarded June 29, 2010; Structured material for the production of hydrogen

Awards

Proctor and Gamble Award in Applied and Environmental Microbiology to Caroline Harwood
Peter Greenberg was recipient of the Doctor of Science Honoris Causa, June 13, 2013, from the University of Guelph, Ontario, Canada.

Graduate Students

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Names of Post Doctorates

NAME	PERCENT_SUPPORTED
Nathan Ahlgren	0.20
Hidetada Hirakawa	0.00
FTE Equivalent:	**0.20**
Total Number:	**2**

Names of Faculty Supported

NAME	PERCENT_SUPPORTED	National Academy Member
Caroline S. Harwood	0.05	Yes
Everett Peter Greenberg	0.02	Yes
FTE Equivalent:	**0.07**	
Total Number:	**2**	

Names of Under Graduate students supported

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Student Metrics

This section only applies to graduating undergraduates supported by this agreement in this reporting period

The number of undergraduates funded by this agreement who graduated during this period: 0.00

The number of undergraduates funded by this agreement who graduated during this period with a degree in science, mathematics, engineering, or technology fields:..... 0.00

The number of undergraduates funded by your agreement who graduated during this period and will continue to pursue a graduate or Ph.D. degree in science, mathematics, engineering, or technology fields:..... 0.00

Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale):..... 0.00

Number of graduating undergraduates funded by a DoD funded Center of Excellence grant for Education, Research and Engineering:..... 0.00

The number of undergraduates funded by your agreement who graduated during this period and intend to work for the Department of Defense 0.00

The number of undergraduates funded by your agreement who graduated during this period and will receive scholarships or fellowships for further studies in science, mathematics, engineering or technology fields:..... 0.00

Names of Personnel receiving masters degrees

NAME
Total Number:

Names of personnel receiving PHDs

NAME
Total Number:

Names of other research staff

NAME	PERCENT_SUPPORTED
Amy Schaefer	0.40
FTE Equivalent:	**0.40**
Total Number:	**1**

Sub Contractors (DD882)

Inventions (DD882)

Scientific Progress

1. Statement of the problems to be studied.

Quorum sensing is a term used to describe bacterial cell-to-cell communication that allows cell-density-dependent gene expression. There are many different types of quorum sensing-regulated functions, but some examples are antibiotic production, biofilm formation and production of tissue destructive enzymes. Many gram-negative bacteria use acyl-HSL signals for quorum sensing. LuxI proteins synthesize these signals. Until recently, all the signals known were fatty acyl-HSLs. These signals bind to LuxR-type receptors that control the expression of specific sets of genes. A few years ago we discovered a non-fatty acyl-HSL produced by the photosynthetic soil bacterium Rhodopseudomonas palustris. This quorum sensing signal is p-coumaroyl-homoserine lactone (pC-HSL), which is synthesized by the RpaI protein. Rather than using a fatty acyl group derived from cellular fatty acid metabolism, RpaI uses an environmental source of a monomeric constituent of plant lignin, p-coumarate, for signal synthesis.

Our goal for this project was to complete a first set of fundamental studies of this new microbial communication system. We are also interested in identifying other non-fatty acid acyl-HSL systems in other bacteria.

2. Summary of the most important results.

In the project we made significant progress on three fronts.

1. We published a study characterizing the pC-HSL responsive transcription factor RpaR (Hirakawa et al, J. Bacteriol. 193: 2598-2607). This involved purifying the RpaR protein, characterizing its pC-HSL-binding activity and determining the DNA sequence to which it binds. We also developed RNAseq methods to identify genes whose transcription is controlled by RpaR.

2. We published a study characterizing an antisense RNA that modulates pC-HSL quorum sensing. (Hirakawa et al., Proc. Natl. Acad. Sci. 109:12141-6) In the course of using RNAseq methods to analyze the influence of quorum sensing on the transcriptome of R. palustris, we found that the most strongly RpaR-activated RNA was an rpaR antisense transcript. This cis-RNA is approximately 300-450 bases in length. Transcription of the rpaR cis-RNA depends on pC-HSL and RpaR and an RpaR-binding site. We used a plasmid to over express the cis-RNA and showed that over expression reduced RpaR levels, rpaI expression and pC-HSL production. We conclude that the cis-RNA inhibits rpaR translation, and this results in suppression of RpaR-dependent rpaI expression and thus pC-HSL production. We presume that the cis-RNA is functioning via base pairing with rpaR transcripts. The cis-RNA represents a new layer of regulation that can be brought to bear on the activity of a LuxR-type transcription factor and represents an example of an antisense RNA where a clear function has been established.

3. We published a study describing a second aryl-HSL signal, cinnamoyl-HSL, produced by a photosynthetic Bradyrhizobium species. (Algren et al., Proc. Natl. Acad. Sci., 108:7183-7188). This molecule differs from pC-HSL in that there is not a hydroxyl group on the aromatic ring. A surprising feature of the cinnamoyl-HSL-directed photosynthetic Bradyrhizobium quorum sensing system is that operates with cinnamoyl-HSL at 1000-fold lower concentrations (picomolar) than do other quorum sensing systems and thus is ultrasensitive. At the same time this system can respond to noncognate acyl-HSLs in the range of nanomolar to millimolar. This is within the range of signals produced by cultures of other bacteria. This raises the possibility that in certain soil habitats the Bradyrhizobium might respond to signals produced by other species (be able to eavesdrop), while at the same time avoiding detection by other bacteria.

3. Bibilography.

Hirakawa H, Y. Oda, S. Phattarasukol, C. D. Armour, J. C. Castle, C.K. Raymond, C. R. Lappala, A. L. Schaefer, C. S. Harwood and E. P. Greenberg. 2011. Activity of the Rhodopseudomonas palustris p-coumaroyl-homoserine lactone responsive transcription factor RpaR. J. Bacteriol. 193: 2598-2607.

Ahlgren, N. S., C. S. Harwood, A. L. Schaefer, E. Griaud, and E. P. Greenberg. 2011. Aryl-homoserine lactone quorum sensing in stem-nodulating photosynthetic bradyrhizobia. Proc. Natl. Acad. Sci. USA 108:7183-7188

Hirakawa H., A. L. Schaefer, E. P. Greenberg and C. S. Harwood. 2012. Anaerobic p-coumarate degradation by Rhodopseudomonas palustris and identification of CouR, a MarR repressor protein that binds p-coumaroyl-CoA. J. Bacteriol. 194: 1960-1967.

Hirakawa, H., C. S. Harwood, K. B. Pechter, A. L. Schaefer and E. P. Greenberg. 2012. An antisense RNA that affects Rhodopseudomonas palustris quorum-sensing signal receptor expression. Proc. Natl. Acad. Sci. USA. 109:12141-6.

Technology Transfer